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**JOHN P. BAPTIST**

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## Uptake of Mixed Fission Products by Marine Fishes

JOHN P. BAPTIST

Bureau of Commercial Fisheries<sup>1</sup> Radiobiological Laboratory, Beaufort, North Carolina

### ABSTRACT

Laboratory experiments were conducted to measure uptake of fission products by fish from water and from oral doses. Availability of fission products to the fish was curtailed considerably by the formation and settling of radioactive particles in the water and by sorption to abiotic surfaces. Spot, *Leiostomus xanthurus*, and Atlantic croaker, *Micropogon undulatus*, accumulated relatively low levels of radioactive material from seawater. Following a single oral dose, croaker assimilated a maximum of 14% in 4 hours, but 94% of the original dose was eliminated after 4 days. Bluefish, *Pomatomus saltatrix*, assimilated only 3% of the cumulative dose of mixed fission products administered in shrimp meat on alternate days for 55 days. Of the amount assimilated from oral doses by croaker and bluefish, the largest concentrations were found in the internal organs.

### INTRODUCTION

Considerable interest has been centered on investigations of the uptake of radioactive materials by fish and other organisms after the release of these materials into the aquatic environment. Most of the data on uptake of fission products by marine organisms have been obtained from radiological surveys of the environment. An extensive account of the surveys conducted in the Pacific atomic-bomb-testing area by the Radiation Laboratory of the University of Washington has been published by Hines (1962). Other reports on both experimental and field investigations appear in Radioecology (Schultz and Klement, 1963). A major share of the experimental work pertains to the uptake of individual radionuclides, rather than mixtures, by various aquatic organisms. Among the few reports on experimental work with fission product mixtures are those by Gong et al. (1957), who measured the uptake of radionuclides by clams from a 2-month-old mixture of fission products, and by Rice, Baptist, and Price (1964), who conducted experiments on

uptake by plankton, shellfish, shrimp, and fish.

To cope with the problem of release of radioactive materials into the aquatic environment, much more experimental work is needed on the uptake of fission products—both mixtures and individual radionuclides. The difficulties which may be encountered in working with the mixtures can readily be understood when one realizes that fission of U-235 in a reactor produces as many as 80 primary fission products, many of which decay into numerous daughter products ranging in mass numbers from 72 to 160 (Glasstone and Sesonske, 1963). The composition of these mixtures changes with time because of the varying physical half-lives of the individual isotopes, which range from a fraction of a second to many years. The purpose of the present investigation was to measure uptake of fission products by fish from seawater and from gastro-intestinal absorption of orally administered fission products.

### METHODS

Uptake of fission product mixtures from seawater was studied in spot, *Leiostomus xanthurus* (15 to 32 grams) and Atlantic

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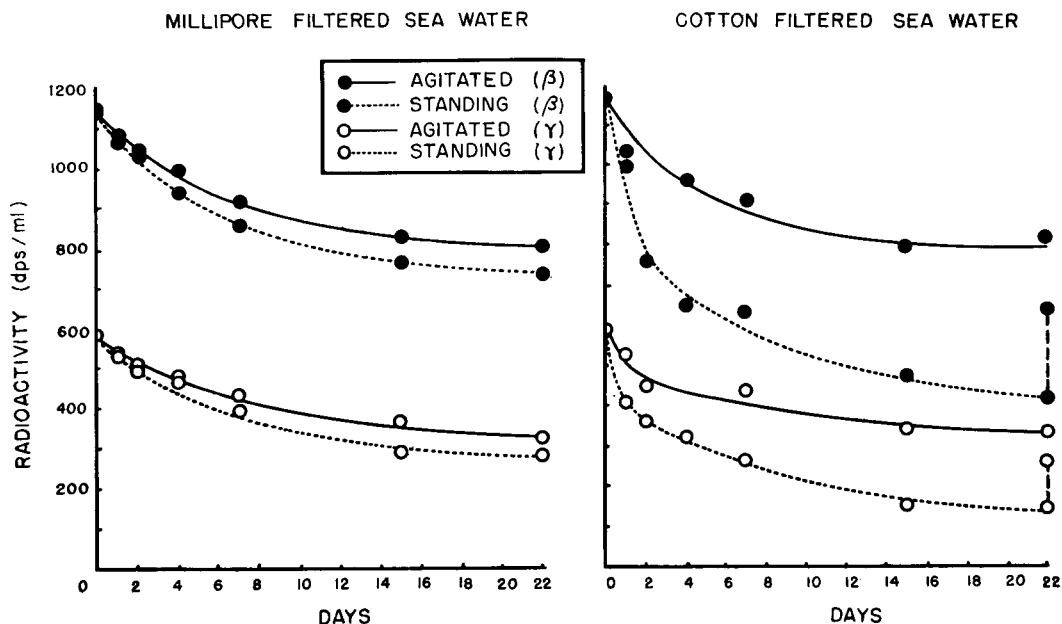


FIGURE 1.—Loss of radioactivity near the surface of Millipore-filtered and cotton-filtered seawater due to formation of particles and surface adsorption of fission products. Cotton-filtered standing water was mechanically agitated for 0.5 hour on the 22nd day (vertical dashed lines).

croaker, *Micropogon undulatus* (66 to 114 grams). Uptake from orally administered doses by croaker and bluefish, *Pomatomus saltatrix* (273 to 574 grams) was also determined. All fish used in these experiments were collected near Beaufort, North Carolina. The experiments were conducted at various times during a 2-year period, so that the fission product mixtures varied considerably in age. The fission products, obtained from the Oak Ridge National Laboratory, were in a 3N nitric acid solution and had decayed less than 45 days when shipped.

Since many fission products are known to be particulate in seawater (Greendale and Ballou, 1954), a test for particle formation was conducted with four 1-liter glass-stoppered Erlenmeyer flasks containing equal amounts of a 248-day-old mixture of fission products in seawater. In order to measure the formation of radioactive particles, it was necessary to first remove particles already present by Millipore-filtering the water in two flasks and cotton-filtering the water in the remaining two flasks. Samples of 1 ml were pipetted from a few millimeters below the surface in each flask and measured for

radioactivity. Samples to be measured for beta ( $\beta$ ) activity were prepared by drying the sample in a planchet. Those to be measured for gamma ( $\gamma$ ) activity were pipetted into vials. Before pipetting each sample, one flask of Millipore-filtered water and one of cotton-filtered water were vigorously agitated for 5 minutes, but the two remaining flasks were not disturbed.

To determine the uptake of fission products from seawater, croaker and spot were kept in separate tanks containing 50 liters each of radioactive seawater and subsequently analyzed for radioactivity. The fission product concentration was adjusted to 1,000 beta disintegrations per second (dps) per gram of water in each tank. The ages of the fission product mixtures were 186 days in the croaker experiment and 68 days in the spot experiment. Seawater first was filtered through cotton to remove large particles which might have provided an excess of surface area for sorption of fission products. The seawater had a salinity of 32 to 35‰, a temperature of 22 to 26 C, a pH of 7.71 to 7.98, and was aerated continuously.

Gastro-intestinal absorption of fission prod-

ucts by croaker and bluefish from single doses and by bluefish from repeated doses were measured in separate experiments. In the single-dose experiments,  $0.5\text{-}\mu\text{C}$  (beta) doses of a 450-day-old mixture were deposited in the stomachs of croaker with a micropipette, and gelatin capsules containing a 216-day-old mixture were inserted into the stomachs of bluefish. In the repeated-dose experiment,  $0.017\text{ }\mu\text{C}$  (beta) and  $0.007\text{ }\mu\text{C}$  (gamma) doses of a 1-year-old mixture were pipetted into gelatin capsules which contained cracker crumbs to absorb the liquid and prevent softening of the gelatin. Each capsule then was inserted into an incision in a dead shrimp tail. The "spiked" shrimp, freshly prepared for each feeding, were randomly distributed in the tank on alternate days for a total of 28 feedings (Table 1). After the first fish sample at 10 days indicated that very little radioactivity had been accumulated, the quantity of fission products in each dose was increased fivefold (beta= $0.085\text{ }\mu\text{C}$ , and gamma= $0.035\text{ }\mu\text{C}$ ).

Each sample consisted of three fish, which were killed by puncturing the brain, homogenized with an equal weight of distilled water. Aliquots of the homogenates were then processed as described below for radioactivity measurements. Samples of various tissues were removed from some of the fish for radioactivity measurements, before the remaining carcass was homogenized. The fission products present in the fish after removal of the digestive tract were considered to have been assimilated. Tissues and 1- to 2-g portions of homogenized carcass were digested in warm nitric acid in 50-ml beakers, transferred to 10-ml volumetric flasks, and made up to volume with distilled water. Aliquots of 5 ml each were evaporated to dryness in plastic dishes and measured for gross beta activity. Aliquots of 2 ml each were pipetted into glass vials and measured for gross gamma activity.

Gross beta activity was measured with a Geiger-Müller tube having an efficiency of 7.7% as calibrated with a  $\text{Ti}^{204}$  standard. Gross gamma activity was measured with a well-type scintillation detector having an efficiency of 31.0% based on a  $\text{Cs}^{137}$  standard.

TABLE 1.—*Summary of repeated doses of a fission product mixture in shrimp meat fed to bluefish on alternate days*

Elapsed days	Cumulative dose (microcuries) per fish	
	Beta	Gamma
9	0.45	0.20
21	2.83	1.62
31	4.74	2.81
43	6.99	4.20
55	10.32	6.29

Both standards were obtained from the National Bureau of Standards. Identical conditions of geometry were maintained for all samples counted in either system. Measurements of the fission product stock indicated a slow rate of decay, so no correction was made during the relatively short time the experiments were conducted. Results are expressed either as disintegrations per second or as percentages of the dose.

#### PARTICULATE NATURE OF FISSION PRODUCTS IN SEAWATER

The uptake of fission products by aquatic organisms depends, in part, upon the availability of the fission products. In the aquatic environment these products may be available to fish from the water and from the organisms which comprise their food. Since availability is governed by the physical-chemical form of the fission products, particle formation in seawater by one of the mixtures was examined prior to conducting the uptake experiments.

Rapid settling of radioactive particles in both Millipore- and cotton-filtered seawater was indicated by the decrease of beta and gamma radioactivity at the surface of the standing seawater (Figure 1). The decrease in radioactivity in the agitated water was attributable entirely to sorption of fission products on the surfaces of the container, since the activity in suspension or solution was distributed homogeneously when sampled. Settling of radioactive particles occurred much more rapidly in the cotton-filtered seawater than in the Millipore-filtered water. Apparently both beta and gamma activity became associated with particles already present, and the larger particles settled more rapidly. As may be surmised from the decrease of radioactivity near the surface of the Millipore-

TABLE 2.—*Assimilation and loss of beta and gamma radioactivity from the digestive tract of croaker with time, expressed as percentage of a single dose of fission products*

Fate of radio-activity	Elapsed time (hours)							
	4		24		48		96	
	Beta	Gamma	Beta	Gamma	Beta	Gamma	Beta	Gamma
Assimilated	14.1	12.5	7.3	5.6	3.6	3.2	5.0	5.6
Digestive tract and contents	72.0	79.0	9.5	5.9	6.1	0.9	0.8	1.3
Lost	13.9	8.5	83.2	88.5	90.3	95.9	94.2	93.1

filtered seawater, the fission products, in addition to becoming sorbed onto the glass surfaces, either formed particles or became associated with new particles being formed.

In both types of filtered water, larger percentages of gamma emitters than beta emitters were removed from the surface layers by sorption and settling (Figure 1). In Millipore-filtered water settling and sorption reduced the initial gamma activity at the surface 52% and beta 41%. In cotton-filtered water the initial gamma activity was reduced 76% and beta 64%. Both flasks of cotton-filtered water that had not been agitated previously were agitated mechanically for 30 minutes and resampled on the 22nd day. A comparison of the surface radioactivity in the water which had been standing for 22 days and then agitated, and that of the water agitated periodically, indicated that the vigorous agitation did not increase the radioactivity in the standing water to the same level as that in the regularly agitated water. This indicated that more radioactivity was firmly fixed to the surfaces of the container of standing water.

#### UPTAKE FROM SEAWATER

Uptake of 68-day-old fission products by spot and uptake of 186-day-old fission prod-

ucts by croaker are shown in Figure 2. Neither species reached a very high concentration. Activity of the seawater was rapidly reduced in both experiments, but more rapidly in the croaker experiment, involving the older fission product mixture. Undoubtedly much of the activity loss was due to sorption of some radionuclides in the mixture to the surfaces of the tank. This sorption process occurred even though the seawater was agitated constantly by aeration and by the swimming movements of the fish.

#### UPTAKE FROM A SINGLE ORAL DOSE

A relatively small proportion of orally administered fission products was taken up by gastro-intestinal absorption in croaker. The maximum amount was assimilated 4 hours after administration (Table 2). Elimination occurred rapidly, 83 to 88% of the dose being lost within 24 hours. The low proportion of assimilation and the rapid loss rate indicate that a large portion of the fission products probably was particulate. After 96 hours, 93 to 94% of the dose had been lost.

Greatest concentration of the assimilated fission products was in the internal organs (Table 3). The remainder of the fish (muscle, bone, skin, scales, eyes, nervous system, and

TABLE 3.—*Uptake of beta and gamma radioactivity with time by tissues of the croaker following a single dose of fission products (expressed as dps/g)*

Tissue	Elapsed time (hours)							
	4		24		48		96	
	Beta	Gamma	Beta	Gamma	Beta	Gamma	Beta	Gamma
Skin	23	10	88	36	18	12	5	3
Muscle	15	13	86	44	15	11	33	26
Internal organs <sup>1</sup>	180	151	231	105	171	62	160	82
Bone	60	14	144	52	74	16	65	11
Carcass	123	58	50	21	23	14	28	21

<sup>1</sup> Excluding the digestive tract.

TABLE 4.—Assimilation and loss of beta emitters from the digestive tract of bluefish with time, expressed as percentage of a single dose of fission products

Fate of radio-activity	Elapsed time (hours)				
	½	1	2	4	8
Assimilated	0	6	2	3	1
Digestive tract and contents	100	90	76	15	3
Lost	0	4	22	82	96

fins) concentrated very little of the radioactive material. The ratio of beta to gamma activity in the different tissues varied considerably without a definite pattern. For example, the beta-gamma ratio in muscle tissue was approximately 1 : 1 after 4 hours and 2 : 1 at 24 hours. After 96 hours, the beta-gamma ratio of bone was approximately 6 : 1, probably due to selective uptake of strontium 90. Because of the complexity of the fission product mixture, however, it would be impractical to attribute much significance to the changes in the beta-gamma ratio in the various tissues. Metabolic usefulness, turnover, translocation, physical decay, and the physical state of the individual radionuclides could have contributed to these changing ratios (beta-gamma ratio of the dose was 1.3 to 1.0). Uptake of beta and gamma radioactivity by bluefish was similar to that of croaker. Bluefish assimilated a maximum of 6% of the dose in 1 hour and excreted 96% after 8 hours (Table 4).

#### UPTAKE FROM REPEATED ORAL DOSES

Since the previous experiments demonstrated that only a small portion of the fission products was concentrated by fish from single doses, this experiment was designed to test whether or not repeated doses would affect the levels of concentration in bluefish tissues. Measurements of uptake in each sample were compared with the cumulative dosage at the time the sample was taken.

The maximum percentage of assimilation of the cumulative doses occurred on or before 10 days, when 7% of the beta and 8.3% of the gamma emitters were assimilated (Table 5). Less than 1% of the cumulative dose was in the digestive tract at each sampling time. At the end of 55 days, 95.9% of the cumula-

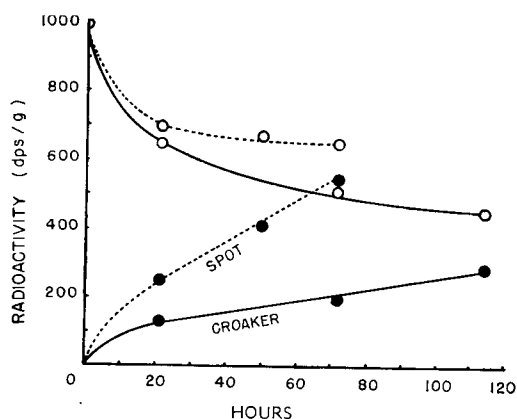


FIGURE 2.—Uptake of fission products by spot and croaker from seawater. (● uptake by fish, ○ loss from water in the corresponding experiments.) Data for croaker from Rice, Baptist, and Price (1964).

tive gamma dosage and 96.8% of the cumulative beta dosage had been lost. These results, on a percentage basis, are not greatly different from those in the single-dose experiments.

Uptake of radioactivity by the various tissues occurred at a slow rate during the first 10 days but increased considerably thereafter as a result of the increased doses (Figure 3). Of the tissues tested, kidney and liver attained the highest concentrations of both beta and gamma emitters. Differences among the concentrations in the other tissues were only slight. Although approximately 1.5 times more beta than gamma radioactivity was available (Table 1), beta concentration in most tissues was only slightly higher than gamma, except on the 55th day. Since rate of decay of beta and gamma emitters was approximately equal in the fission product stock, it may be assumed that there was some preferential uptake of gamma emitters.

#### DISCUSSION

Fish accumulate relatively small amounts of fission products, as indicated by the present experiments and the published reports of radiological surveys in the natural environment (Welander, 1955; Donaldson, 1960; Berner et al., 1962). Among the reasons for this are that most fission products are not essential to metabolism and that many, being

TABLE 5.—Assimilation and loss of beta and gamma radioactivity from the digestive tract of bluefish with time, expressed as percentage of a cumulative dose of fission products<sup>1</sup>

Fate of radio-activity	Elapsed time (days)							
	10		31		43		55	
	Beta	Gamma	Beta	Gamma	Beta	Gamma	Beta	Gamma
Assimilated	7.0	8.3	3.7	4.5	2.8	3.8	3.0	3.4
Digestive tract and contents	0.3	0.6	0.6	0.8	0.3	0.6	0.2	0.7
Lost	92.7	91.1	95.7	94.7	96.9	95.6	96.8	95.9

<sup>1</sup> See Table 1.

particulate in seawater, cannot pass through living membranes. Another reason is that naturally occurring elements with metabolic properties similar to some fission products are abundant in seawater (for example, K vs. Cs<sup>137</sup>) and therefore are taken up more readily. Particulate elements probably are adsorbed to a slight extent on fish but to a great extent on phytoplankton. Different species of phytoplankton concentrated Ce<sup>144</sup> from 2,000 to 4,500 times that in seawater, and the particulate form was taken up at a faster rate than the ionic (Rice and Willis, 1959).

The high concentration of fission products by phytoplankton probably accounts indirectly for the presence of radioactivity in fish in many reported instances. Since fish accumulate only small amounts of fission products directly from seawater, where the radioactivity is quickly diluted and dispersed in the environment, it is likely that accumu-

lation is predominantly from the food chain. Results from operation "Wigwam" demonstrated no significant concentrations of radioactive materials in fishes except in the stomach and gut regions (Berner et al., 1955), indicating that uptake was from ingested water or food, or both. Some radioisotopes that occur in the particulate form in seawater have been found in the internal organs of fish, birds, and other animals (Palumbo, 1961). Palumbo therefore postulated that these radioelements must have been converted to the soluble form at some point in the food cycle. This appears feasible, since the solubility of most fission products is affected by the pH. I have demonstrated, for example, that 6.3% of the Ce<sup>144</sup>—Pr<sup>144</sup> in a solution at pH 8.5 passed through a dialysis membrane, 29.5% in a solution at pH 7, and 57.5% in a solution at pH 2. I also have measured the pH of stomachs of 10 croaker 20 hours after feeding and found the mean value to be pH 3.35, which is within

TABLE 6.—Relative radioactivity contributed by fission products of biological interest at different times after slow-neutron fission of U<sup>235</sup>

Isotope	Physical half-life	Percentage of total radioactivity <sup>1</sup>				Percentage ionic in seawater <sup>2</sup>	Percentage absorbed on ingestion <sup>3</sup>
		70 days	200 days	250 days	438 days		
Sr <sup>90</sup>	54 days	10.1	7.8	6.1	1.4	87	30
Sr <sup>90</sup> —Y <sup>90</sup>	28 years,						
	64 hours	<1.0	<1.0	<1.0	2.5	87	30
Y <sup>91</sup>	58 days	11.9	10.2	8.5	2.0	0	—
Zr <sup>95</sup>	65 days	13.6	14.1	12.2	4.9	1	0.01
Nb <sup>95</sup>	35 days	14.0	25.0	23.0	10.2	0	0.01
Ru <sup>103</sup> —Rh <sup>103</sup>	41 days,						
	57 hours	7.5	3.7	2.3	<1.0	0	20
Ru <sup>106</sup> —Rh <sup>106</sup>	1 year,						
	2 hours	<1.0	1.1	1.5	2.8	0	20
Cs <sup>137</sup> —Ba <sup>137</sup>	30 years,						
	2.6 min	<1.0	<1.0	<1.0	2.0	70	100
Ba <sup>140</sup>	13 days	3.7	—	—	—	—	5
La <sup>140</sup>	40 hours	4.0	—	—	—	—	—
Ce <sup>141</sup>	32 days	10.0	1.9	<1.0	—	2	0.01
Pr <sup>143</sup>	14 days	4.7	—	—	—	—	—
Ce <sup>144</sup> —Pr <sup>144</sup>	282 days,						
	17 min	4.7	13.1	16.9	30.0	2	0.01
Pm <sup>147</sup>	2.6 years	<1.0	2.2	3.1	7.4	—	0.01

<sup>1</sup> Adapted from data of Hunter and Ballou (1951).<sup>2</sup> Greendale and Ballou (1954).<sup>3</sup> Langham (1960).

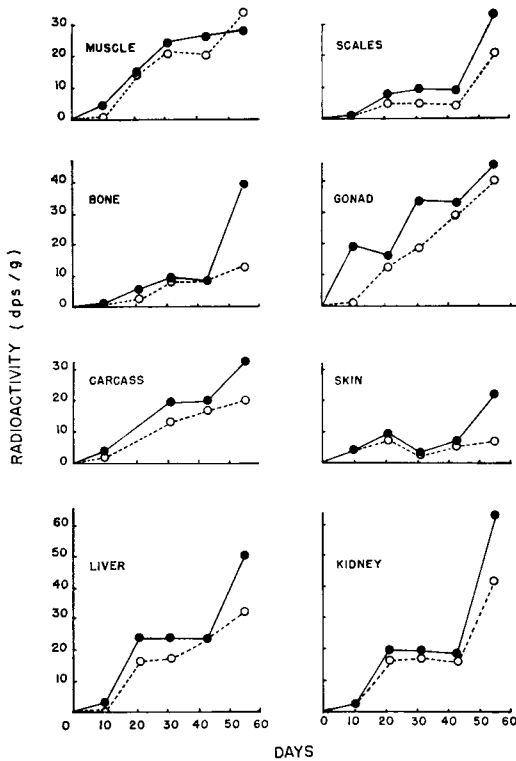


FIGURE 3.—Uptake of radioactivity by bluefish tissues from doses of a fission product mixture given in food on alternate days. (● beta emitters; ○ gamma emitters.)

the range reported for other fishes (Brown, 1957).

Although radionuclides accumulated by the fish in these experiments were not identified, their presence can be surmised by considering characteristics of the individual radionuclides and factors affecting their accumulation. Radioisotopes which represent a small proportion of the mixture, have a short physical half-life, occur as particles in seawater, or are not absorbed after ingestion are not likely to be accumulated to any great extent. By ruling out the isotopes in Table 6 that have combinations of the above factors, it is possible to select the isotopes most likely to have been accumulated. For the 450- and 365-day-old mixtures these would include  $\text{Sr}^{90}$ — $\text{Y}^{90}$ ,  $\text{Ru}^{106}$ — $\text{Rh}^{106}$ ,  $\text{Cs}^{137}$ — $\text{Ba}^{137}$ ,  $\text{Ce}^{144}$ — $\text{Pr}^{144}$ , and  $\text{Pm}^{147}$ . These isotopes would apply also for the 186-, 216-, and 248-day-old mixtures along with  $\text{Zr}^{95}$ ,  $\text{Nb}^{95}$ , and  $\text{Ru}^{106}$ — $\text{Rh}^{106}$ . For the

68-day-old mixture all the isotopes in Table 6 would be included except possibly  $\text{Ba}^{140}$ ,  $\text{La}^{140}$ , and  $\text{Pr}^{143}$ .

The published literature appears to bear out these assumptions. Rice, Baptist, and Price (1964) demonstrated the presence of  $\text{Ce}^{144}$ — $\text{Pr}^{144}$  in internal organs and  $\text{Sr}^{90}$ — $\text{Y}^{90}$  in bone of croaker by measuring the transmission of beta particles through aluminum absorbers. Aside from neutron-induced isotopes, the only significant concentrations of fission products in minnows in the Columbia River were  $\text{Cs}^{137}$  in muscle and  $\text{Sr}^{89,90}$  in bone (Davis et al., 1958). Flounder collected near the Windscale Plant in England contained  $\text{Sr}^{90}$  and  $\text{Ru}^{106}$ — $\text{Rh}^{106}$  (Dunster, 1958). In the Pacific bomb-testing areas, fish contained relatively small amounts of  $\text{Sr}^{89,90}$ ,  $\text{Cs}^{137}$ ,  $\text{Ru}^{106}$ — $\text{Rh}^{106}$ ,  $\text{Zr}^{95}$ ,  $\text{Ce}^{141}$ , and  $\text{Ce}^{144}$ — $\text{Pr}^{144}$ , but the largest proportion of radioactivity was attributable to  $\text{Zn}^{65}$ , a neutron-induced radionuclide (Rinehart et al., 1955; Thomas, Reid, and Lust, 1958). Clams accumulated fission products to levels ranging from a concentration factor of 1 for  $\text{Ru}^{103,106}$  and  $\text{Sr}^{89}$  to 13 for  $\text{Zr}^{95}$  and 280 for rare earth elements (Gong et al., 1957).

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